OSMOTIC PROPERTIES OF THE ERYTHROCYTE

IV. Is the Permeability of the Erythrocyte to Water Decreased by Narcotics?

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I

In the course of studies connected with his well-known "Haftdruck" theory, Traube (1908) made the observation that certain lipoid-soluble substances, such as amyl alcohol, which are also known to possess narcotic properties, may, under appropriate conditions, exert an antihemolytic effect. This effect he interpreted as being due to "eine Verdickung der Lipoidschicht welche die Stabilität der Blutkörperchen gegen andere Hämolytika ändern muss." A few years later Arrhenius and Bubanović (1913) obtained similar results with chloroform, ethyl ether, ethyl and amyl alcohols, and benzene. As an example of these results one typical experiment with a hypotonic solution containing chloroform may be cited. In this experiment it was found after one hour at 37° C., followed by several more in the ice-box, that the percentage of hemolysis decreased from 60 per cent in the absence of chloroform to a minimum of 37 per cent in the presence of 0.2 per cent, rising again at higher concentrations. Arrhenius and Bubanović did not hesitate to conclude that "diese Wirkung beruht vermutlich auf einer Verlangsamung des Eindringens von Wasser in die Zellen." Among the more recent workers in this field, Yoshitomi (1920) found after exposures of 2 to 18 hours to hypotonic solutions, a lesser degree of hemolysis in the presence of certain concentrations of ether, chloroform, chloretone and amylene hydrate than in their absence. He suggested that this effect might be due either to a less ready entrance of water into, or a more ready escape of salts from, the cells. Jarisch (1921) also obtained an inhibition of osmotic hemolysis with alcohol, ether, amylene hydrate and urethane. While he did not state in very precise terms his conception of the antihemolytic action of these substances, his use in this connection of the term "wasserhemmend" would seem to indicate that his views were not unlike those of Arrhenius and Bubanović.

Since these and similar results obtained with the erythrocyte have frequently been cited in support of the view that narcosis is associated with a decreased cell permeability, it seems necessary for us to point out that such evidence is entirely inconclusive. In the first place, osmotic hemolysis is a complicated process involving not only (*a*) the entrance of water into the erythrocyte, but (*b*) the escape of hemoglobin, (*c*) the possible loss from the cell of salts and other osmotically active substances (see in this connection Ponder and Saslow, 1931) as well as (*d*) a variety of possible changes of diverse nature, not directly associated with permeability, in what is commonly and rather loosely called the "osmotic resistance" of the cell. While it is undoubtedly true that anything that decreases the permeability of the cell to water (factor *a*) will, in general, tend to delay osmotic hemolysis, the reverse statement is by no means true. A delay might equally well be produced by the operation of any or all of factors *b*, *c*, and *d*.

A more fundamental objection, however, to evidence of the type mentioned above, is that it is very unlikely that the investigators in question obtained from their experiments any real information as to the *rate* of hemolysis, which is the thing of greatest importance in connection with questions of permeability to water. It is, of course, conceivable that if at the end of some single arbitrarily selected time a lesser degree of hemolysis is obtained in the presence than in the absence of a narcotic, the difference might be due to a slower rate of progress in the former case towards the same final end state of the system. On the other hand, the possibility must be considered that the narcotic exerts its effect primarily on the degree of hemolysis ultimately attained; in that case, with no knowledge of the position of equilibrium towards which the system is proceeding—or which it may indeed have reached at the time the observation is made—no valid conclusions whatever can be drawn as to the fundamental rate of the hemolytic process.

Though this principle would seem to be a self-evident one, it has been very frequently disregarded in the past, not merely in studies on hemolysis, but in other fields of physiological work as well. It should be most strongly emphasized, therefore, that while it is possible to study a position of equilibrium with no exact knowledge of the rate at which it is attained, the reverse procedure of attempting to draw conclusions concerning a rate, with no information whatever as to the end state which the given system is approaching, is entirely unwarranted and can lead only to confusion (see in this connection Jacobs, 1928). In the present paper the distinction between the effects of certain urethanes on the "equilibrium" and the "rate" factors concerned in osmotic hemolysis will be illustrated; and it will further be pointed out that since

as far as we are aware the work of previous investigators in this field makes no such distinction, it can be expected to throw no real light on the question of the permeability of the erythrocyte to water.

II

As a first step in the separation of the two types of factors, it seemed important to obtain curves representing the entire course of hemolysis from its beginning until further change had ceased. This necessary type of information, which, as has been mentioned, has apparently not been supplied by previous workers, is particularly easy to obtain by the method of one of the authors (Jacobs, 1930). A typical experiment is represented in Fig. 1 in which the erythrocytes were those of the ox and the narcotic was ethyl urethane. The blood in this, as in all the

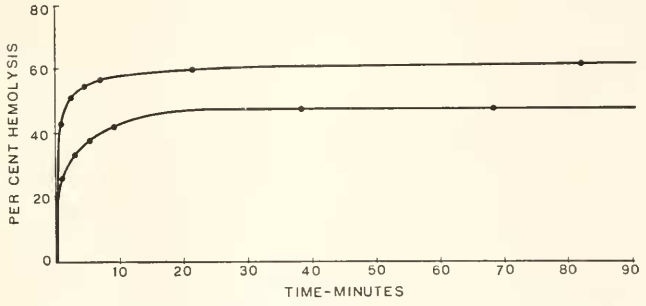

FIG. 1. Course of hemolysis of ox blood in buffered 0.090 M NaCl in the presence (lower curve) and absence (upper curve) of 0.3 M ethyl urethane: pH, 7.4; temperature, 20° C.

other experiments here described, was thoroughly and almost instantaneously mixed with the solutions used in the proportion of approximately 1 to 500. Kymograph records were made of the ensuing hemolysis and from these the curves in the figure were reconstructed. Because of the enormous importance of pH and temperature changes in experiments involving equilibria, the precautions described by Jacobs and Parpart (1931) were employed in all such experiments, the pH being kept almost constant in this case at approximately 7.4, and the temperature at 20° C.

An inspection of Fig. 1 shows very clearly that under the conditions of this experiment the effect of ethyl urethane upon the final end-point reached by the system is far more striking than any possible effect it might have upon the rate of hemolysis as such. Thus, in the absence of the urethane, a degree of hemolysis of approximately 61 per cent was attained in between 20 and 30 minutes, and after that time no further

change occurred; in its presence a final degree of hemolysis of 48 per cent was reached in about the same time, and this likewise underwent no further change. Very similar results were obtained in a number of other experiments. It is evident, therefore, that no information about the rate of hemolysis can be secured in such cases by observing at the end of some arbitrarily selected time the mere degree of hemolysis that then happens to exist.

It is to be noted that the antihemolytic effect of ethyl urethane is not a simple osmotic one due to the greater total concentration of the solution containing it. Such an effect could be obtained only with a non-penetrating substance, since otherwise the solute molecules would distribute themselves inside and outside the cell in such a way that their osmotic effects would everywhere balance. In the case of the urethanes, however, not only is the penetrating power for cells in general known to be extremely high, but a number of hematokrit measurements made in the course of these experiments showed the absence of any measurable osmotic effects on cell volume. As a matter of fact, though the concentration of the ethyl urethane in this particular experiment was 0.3 M, the antihemolytic effect was only that which would have been produced osmotically by an increase in concentration of possibly 0.006 M in a solution of a non-penetrating non-electrolyte.

As to the effect of ethyl urethane on the fundamental rate of hemolysis, apart from that on the final degree attained, this cannot be determined by a mere inspection of such curves as those in Fig. 1. Even though a measurably longer time were required to attain a given degree of hemolysis in the presence than in the absence of the narcotic, it would be impossible to be certain, without a fairly complicated mathematical analysis of the results, how much of the observed effect was due merely to the shift in the final equilibrium. Since, therefore, even under the relatively favorable conditions provided by the possession of two complete hemolysis curves it is very difficult to draw conclusions about the effect of a narcotic on the rate of the process, what information of value could conceivably be obtained from the knowledge of only a single point on each curve? As a matter of fact, most of the observations mentioned in the introductory paragraph were taken at times long after those at which the final equilibrium must have been attained and could, therefore, by no possibility throw any light upon the rate of hemolysis, and by implication, upon the possible rate of entrance of water into the cells. Though, as will be shown later, the conclusion of previous investigators that the rate of entrance of water into the erythrocyte is slowed by the presence of a narcotic may in itself be entirely correct, it may not validly be drawn from the data they have presented.

Having found in experiments of the type of that represented in Fig. 1 that the final equilibrium condition is usually reached within 20 or 30 minutes, and in any case in less than an hour, it seemed desirable to study by more quantitative methods than those previously used the effects of varying the concentration of the narcotic. In Table I are given the results of one such experiment with ethyl urethane in which the percentages of hemolysis reached in one hour in the presence of different concentrations of this substance were determined. The concentration of NaCl used was selected as a favorable one for the particular sample of ox blood employed. The pH in this case was approximately 7.35 and the temperature, as before, 20° C. It will be noted that as the concentration of urethane increased, the degree of hemolysis decreased until a maximum effect was reached probably somewhere between

TABLE I

Degree of hemolysis attained by ox erythrocytes in one hour in a buffered 0.085 M NaCl solution containing different concentrations of ethyl urethane.
pH, 7.35; temperature, 20° C.

Concentration of urethane	Percentage of hemolysis
—	75
0.0078	75
0.0156	74
0.0313	74
0.0625	70
0.125	70
0.25	68
0.5	73
1.0	100

0.25 M and 0.5 M. Beyond this point a hemolytic effect of the narcotic became evident. The general results of this entirely typical experiment with ethyl urethane do not differ in principle from those obtained by Arrhenius and Bubanović with chloroform, if it be assumed, as was almost certainly the case, that what these investigators measured was the final position of equilibrium of the system.

In another experiment, whose results are given in Table II, the general procedure was reversed by keeping the concentration of urethane constant at 0.1 M and varying that of the hypotonic solution. In this way the antihemolytic effect of the narcotic on a considerable proportion of all the erythrocytes in the blood could be observed. It will be noted that although the results show certain minor irregularities, there is in no case any departure from the previously observed antihemolytic effect. Similar, though less complete, results were also obtained with several other urethanes, as well as with ethyl alcohol. Taking together these results and those reported by previous workers, the evidence

seems to be entirely consistent that the degree of hemolysis ultimately attained in a hypotonic solution may be reduced by a variety of narcotic substances in proper concentrations. This fact, however, though of interest in other ways, throws little or no light upon the question of the effect of narcotics upon the permeability of the erythrocyte to water. What is needed is information not about the final equilibrium but about the fundamental rate at which hemolysis occurs.

III

It has been pointed out in another place by one of the authors (Jacobs, 1928) that in cases where the position of equilibrium of a

TABLE II

Degree of hemolysis attained by ox erythrocytes in one hour in buffered NaCl solutions of different concentrations in the absence and presence of 0.1 M ethyl urethane.
pH, approximately 7.35; temperature, 20° C.

Concentration of NaCl	Percentage of Hemolysis	
	Urethane absent	Urethane present
0.098	82	78
0.099	78	74
0.100	73	69
0.101	71	65
0.102	69	59
0.103	67	55
0.104	63	53
0.105	57	48
0.106	53	44
0.107	50	37
0.108	45	38
0.109	41	33
0.110	34	22

hemolytic system is influenced by the same factor whose effect on the rate of hemolysis it is desired to study, the general rule should be followed of keeping the system at all times as far away from equilibrium conditions as possible. Thus, in investigating the effect of temperature upon the rate of osmotic hemolysis, it was found that where only water or very strongly hypotonic solutions were employed consistent and plausible results could be obtained, while with less strongly hypotonic solutions the results were erratic and at first sight inexplicable. Similarly, in the case of narcotics, which have been shown in the preceding section to affect in a striking manner the degree of hemolysis finally attained, the only satisfactory method of studying their effect on the rate

of hemolysis as such would be to work with very strongly hypotonic solutions, or preferably, with distilled water. Most methods for studying hemolysis are much too slow for use in experiments of this type—which doubtless accounts for the fact that they have apparently not heretofore been made. The method of one of the authors (Jacobs, 1930) is entirely suitable for this purpose, however, and some results obtained with it may now be described.

In Table III are indicated the effects on hemolysis by water of the addition of different amounts of ethyl, n-butyl, i-amyl and phenyl urethanes. Though for such short times the experimental errors are

TABLE III

Effect of Various Urethanes on the Time Required for 75 Per Cent Hemolysis of Ox Blood by Distilled Water

Ethyl Urethane		n-Butyl Urethane		i-Amyl Urethane		Phenyl Urethane *	
Concentration	Time	Concentration	Time	Concentration	Time	Concentration	Time
	seconds		*seconds*		*seconds*		*seconds*
—	1.5	—	1.3	—	1.30	—	1.25
0.0313	1.5	0.0015	1.4	0.0003	1.30	0.00025	1.25
0.0625	1.5	0.0031	1.4	0.0013	1.28	0.0005	1.30
0.125	1.7	0.0062	1.5	0.0025	1.32	0.001	1.30
0.25	2.0	0.0125	1.6	0.005	1.42	0.002	1.35
0.5	2.2	0.025	1.5	0.01	1.50	0.004	1.32
1.0	2.6	0.05	2.1			0.008	1.52

* See also Table V.

relatively large, it will be noted that the results are on the whole entirely consistent and that for each substance there is a slight but unmistakable retardation of hemolysis as the concentration increases. Furthermore, the effectiveness of the different urethanes, as might be expected, proves to be very different. As judged by the dilutions at which a retardation of hemolysis first becomes apparent, the order of effectiveness is:

$$\text{ethyl} < \text{n-butyl} < \text{i-amyl} < \text{phenyl}.$$

This is not only the order in which narcotic effects are usually manifested by these substances, but there is a rough quantitative agreement between the concentrations at which hemolysis begins to be retarded and those found by Dr. E. B. Harvey to be effective in reversibly suppressing the cleavage of the *Arbacia* egg. The latter concentrations, as cited by Lucké (1931), are as follows: ethyl, 0.05 M to 0.2 M; n-butyl, 0.0125 M to 0.05 M; i-amyl, 0.005 M to 0.01 M; and phenyl, 0.00125 M to 0.005 M.

In the case of ethyl urethane, where the effective concentration is fairly high, it is conceivable that at least a part of the delay in hemolysis may be due to osmotic factors. Even a substance that penetrates a cell as rapidly as a urethane might, if sufficiently concentrated, slow to a measurable extent the rate of attainment of the final osmotic equilibrium between the cell and its surroundings, while having no direct effect upon the position of the equilibrium. Whatever may be the validity of this objection in the case of ethyl urethane, however, it is certain that it cannot hold in the case of the other three substances, where the effective concentrations are of the order of 0.01 M to 0.001 M. It has been shown elsewhere by one of the authors (Jacobs, 1932) that even in the case of a completely non-penetrating non-electrolyte such as saccharose, the time required for hemolysis in a 0.01 M solution differs from that in

TABLE IV

Times required for 75 per cent hemolysis of ox blood in NaCl solutions containing different amounts of ethyl urethane. Temperature, 20° C.

Concentration of Urethane	Concentration of NaCl							
	Water	0.02 M	0.04 M	0.06 M	0.07 M	0.073 M	0.076 M	0.08 M
	seconds	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*
——	1.5	4.0	5.7	7.0	10.3	18.0	22.5	70.2
0.0313 M.....	1.5	4.2	5.6	7.5	11.7	19.8	26.8	73.4
0.0625 M.....	1.5	4.0	5.8	7.3	12.5	18.9	34.4	96.5
0.125 M.....	1.7	4.0	6.2	8.3	16.0	27.8	56.5	156
0.25 M.....	2.0	4.7	6.5	9.1	20.3	54	103.5	332
0.5 M.....	2.2	4.8	7.0	9.7	39.0	140	280	832
1.0 M.....	2.6	5.1	7.1	10.3	32.6?	274	317	420

water only to a barely measurable extent, the difference for ox erythrocytes being perhaps 0.1 second. With lower concentrations than 0.01 M of substances of extremely high penetrating power such as the urethanes, direct osmotic effects could certainly not be measured; and any effects that could be measured would therefore necessarily be of a more specific nature. Though the retardation of hemolysis in water by urethanes is apparently always small in amount and the errors of the determinations are relatively large, the results obtained in these and in other experiments have been sufficiently consistent to leave little doubt that the substances in question are able to affect the rate of hemolysis under conditions where any shift in the final theoretical equilibrium is of negligible importance. Such an effect, though erroneously inferred by other workers from their experiments, has not, we believe, previously been demonstrated.

In concluding the presentation of experimental data there may be added in Tables IV and V figures showing the gradual transition from conditions where the fundamental rate factor is primarily concerned in determining the time of hemolysis to those where the equilibrium factor tends to dominate the situation. In the light of the facts presented in the preceding section, it is evident that the striking effects produced by all the substances in the most concentrated salt solutions are due chiefly to a change of the hemolytic end-point in the direction of a reduced final degree of hemolysis. The rate factor as such cannot be studied in such solutions. In the case of ethyl urethane, one additional factor appears in the last figure of the last column, namely, a direct hemolytic effect of

TABLE V

Times required for 75 per cent hemolysis of ox blood in NaCl solutions containing different amounts of phenyl urethane. Temperature, 20° C. Each figure for water and for the lower concentrations of NaCl is the average of four determinations.

Concentration of Urethane	Concentration of NaCl						
	Water	0.02 M	0.04 M	0.06 M	0.075 M	0.08 M	0.085 M
	seconds	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*
——	1.50	3.87	5.17	6.92	11.1	19.8	41
0.0005 M	1.50	—	5.17	6.70	11.8	28.5	46
0.001 M	1.55	3.88	5.23	6.72	12.8	30.0	55
0.002 M	1.52	3.85	5.20	7.05	14.5	76.5	115
0.004 M	1.70	3.90	5.30	7.58	48.5	86.0	—
0.008 M	1.95	3.93	5.38	7.95	54.2	121.0	870

the urethane in very high concentrations; but this factor is of comparatively small importance in connection with the present experiments.

One other point suggested by the results obtained with i-amyl and phenyl urethanes may be mentioned briefly, namely, that whereas the retarding effect of urethanes on hemolysis by water never failed to appear in our experiments, it was sometimes more doubtful in the case of the most dilute salt solutions, *e.g.*, 0.02 M. The experiment chosen for representation in Table V was selected to show this condition, which was, however, not invariably observed. We are unable to suggest a reason why the retarding effect of certain urethanes may at times be more pronounced in the absence than in the presence of salts, but may point out that a somewhat similar but much more striking effect has been observed by Lucké (1931) in the case of the *Arbacia* egg. His explanation is that if the entrance of water into the cell has already been strongly retarded by the presence of salts (especially those of calcium), narcotics are unable to produce any further effect. In the case of the

erythrocyte it will be shown in a later paper that very low concentrations of salts, including NaCl, are effective in some non-osmotic manner in retarding osmotic hemolysis. It is conceivable, therefore, though by no means certain, that a similar principle is involved in the two cases.

IV

It has been shown in the preceding sections that various urethanes in the proper concentrations are able to reduce the degree of hemolysis finally attained in certain hypotonic salt solutions. This increase in the osmotic resistance of the cells, which has been noted by a number of previous observers, is an equilibrium rather than a rate effect and is best seen in solutions whose concentrations are such as to cause the disappearance of some but not all of the erythrocytes in the given sample of blood. It has also been shown that under conditions where complete hemolysis is very rapidly produced and where possible changes in the theoretical position of equilibrium of the system are of negligible importance, narcotics are able to bring about a slight but consistent slowing of the rate of the process. This effect, which is best seen in distilled water and which has frequently been confused with the one first mentioned, has not as far as we are aware, previously been demonstrated, though from the standpoint of cell permeability it is the more important of the two. Its possible nature will be considered after attention has first been given to the more striking and better known change in the osmotic resistance of the cells.

As has been mentioned above, there are at least four different ways in which the osmotic resistance of the erythrocyte might be affected (factors *a*, *b*, *c* and *d* on page 314). Of these factors, the first, namely, a change in permeability to water, may almost certainly be ruled out as a possible cause of any change in the position of final equilibrium of the hemolytic system. Only if the cells at some point became completely impermeable to water could this factor alone do more than change the rate at which the equilibrium is reached; the equilibrium itself would remain unaltered. As a matter of fact there is an abundance of evidence that the erythrocytes do not at any time become completely impermeable to water.

On the other hand, a changed permeability to salts and other osmotically active substances contained within the cell (factor *c*) might conceivably alter its osmotic resistance. Since hemolysis by hypotonic solutions is due to an excess of osmotic pressure within the cell, any escape of materials that reduced this excess would not only slow the rate of hemolysis in all cases, but in certain critical cases would prevent its occurrence altogether. The possibility of a leakage of salts from the

erythrocyte in ordinary osmotic experiments has been emphasized by Ponder and Saslow (1931), and it is by no means inconceivable, or indeed unlikely, on *a priori* grounds that such a leakage might be favored by narcotics. In sufficiently high concentrations these substances tend by destroying the erythrocyte to permit a very ready escape of materials from its interior; and it is entirely possible that in lower concentrations they might injure its surface sufficiently to increase any loss of electrolytes already in progress.

Though this theory has a certain degree of plausibility, it nevertheless seems necessary to discard it in view of the direct evidence obtained by Siebeck (1922) that narcotics actually reduce to an easily measurable extent the rate of exchange of ions between the cell and its surroundings, and that furnished by Joel (1915) that the gradual increase in the electrical conductance of a suspension of erythrocytes is slowed rather than accelerated in the presence of such substances. As far as the available evidence goes, the effect of ordinary concentrations of narcotics would seem, if this factor were of importance, to be in the direction of reducing rather than of increasing the osmotic resistance of the erythrocyte.

Turning next to factor *d*, which involves some change or changes in the osmotic resistance of the cell not associated with permeability factors, the possibility suggested by Traube that the narcotic may produce in some way a thickening or a strengthening of the cell membrane and so oppose hemolysis may first be considered. Such an explanation appears to be an unlikely one in view of the fact that the surface of the erythrocyte seems normally to offer little resistance to osmotic volume changes. (See in this connection Jacobs 1931, 1932.) As a matter of fact, the increased resistance in the presence of, for example, 0.3 M ethyl urethane, which is by no means the greatest effect we have observed, may correspond to a change in the critical concentration of NaCl by 0.003 M, amounting in terms of osmotic pressure to perhaps one-eighth of an atmosphere. That the delicate cell membrane could be strengthened to support this excess of pressure does not seem very likely.

A much more plausible possibility is that the narcotic may in some way have a tendency to cause a diminution in the volume of the cell and so to oppose its swelling in hypotonic solutions. Effects of this sort are already known in the case of other agents. For example, the increased osmotic resistance of erythrocytes in alkaline media and at high temperatures (Jacobs and Parpart, 1931) and in solutions of non-electrolytes (Jacobs, 1932) is probably to be accounted for in this way. As a matter of fact, v. Knaffl-Lenz (1918) has reported a decrease in the volumes of erythrocytes, as measured by the hematokrit, on the addition

of certain narcotics, though the times required to produce this effect in his experiments were much longer than those involved in the present series; and, furthermore, his results were indecisive in the case of the only urethane he used. We have been unable to detect with certainty by the hematokrit method any such differences in volume in the case of ethyl urethane solutions, though in view of the rather large errors of the hematokrit method and the very slight volume changes required to produce a considerable difference in the observed percentage of hemolysis (Jacobs and Parpart, 1931) we do not feel that this possibility has been entirely ruled out.

The last factor that will be discussed is the second of those mentioned above, namely, the escape of hemoglobin from the cell. This factor has frequently been neglected in studies on osmotic hemolysis in the past owing, no doubt, to the old belief that hemolysis is produced by an actual bursting of the cell when the internal pressure has reached a sufficiently high point. If this were the mechanism of hemolysis, then the escape of hemoglobin would, in fact, be an unimportant part of the process. It seems certain, however, from the phenomenon of "reversible hemolysis," so-called, that the cell is not ordinarily ruptured by mild hemolytic agents, but that at a certain time, as a result of stretching or some other change in its surrounding membrane, the latter becomes permeable to the hemoglobin contained within the cell. This permeability to hemoglobin is reached in such a sudden and definite manner that osmotic hemolysis is apparently an "all-or-none" phenomenon, *i.e.*, up to a certain point no hemoglobin escapes from the cell; beyond that point an almost infinitesimal increase in the volume of the cell results in the free outward diffusion of all of its hemoglobin (Saslow, 1929; Parpart, 1931). Unfortunately, we know too little at present about the physical state of the hemoglobin within the cell and the possible effects of changes in this state on its diffusibility. It is usually assumed, however, in the absence of evidence to the contrary, that under all usual conditions we have to do with a simple aqueous solution of hemoglobin and that the possibility of its escape from the cell depends merely on the character of the cell membrane.

The assumption that the escape of hemoglobin from the erythrocyte depends primarily on the cell membrane may or may not be true. It is of interest, however, to see whether it can be made the basis of a plausible explanation of the effect of narcotics on osmotic resistance. There is considerable evidence that at the surface of the erythrocyte in addition to lipoid substances which give to the cell certain of its physical properties (Mudd, S., and E. B. H. Mudd, 1926) and which perhaps determine its free permeability to all lipoid-soluble substances, there are

regions through which water, ions, and non-lipoid-soluble organic substances of low molecular weight can pass. Though the exact structural nature of these regions is not known, they may, at least in a semi-figurative sense, be called "pores." A further discussion of this theory as applied to the erythrocyte is given by Mond and Hoffman (1928) and by Jacobs (1931).

Whatever may be our ideas of the exact nature of the hypothetical "pores" in the cell membrane, it must not be forgotten that certain purely objective facts are well known; namely, that non-lipoid-soluble molecules of sufficiently low molecular weight pass through the wall of the erythrocyte readily, those of higher molecular weight more slowly, and those whose molecular weight (or molecular volume) exceeds a certain size fail to do so at all. The hemoglobin molecule, of course, enormously exceeds the critical size for penetration. Nevertheless, in osmotic hemolysis a point is somewhere reached where rather suddenly the cell becomes permeable to hemoglobin. Without attaching too literal a meaning to the statement, we may say that at this point the "pores" have been enlarged sufficiently to permit the escape of this molecule.

Now we have a certain amount of experimental evidence that narcotics are able—presumably by adsorption—to diminish the size of the pores in artificial membranes, or, at any rate, to render more difficult the passage of certain substances through these membranes (Anselmino, 1928 *a*, *b*). Suppose that the same were true of the erythrocyte at the point where it undergoes hemolysis. In this case, the presence of a sufficient concentration of a narcotic substance might be expected to convert a "pore" that would otherwise just permit the passage of hemoglobin into one that would just fail to permit it. Further swelling would be necessary to cause hemolysis. The osmotic resistance of the cell would thereby be raised, just as it is known to be in fact. Furthermore, the effectiveness of weakly adsorbed narcotics would be less than that of strongly adsorbed ones and, again, there is a parallel between the adsorbability of different urethanes and their ability to prevent hemolysis.

Accepting in a purely tentative manner this explanation of the effect of narcotics upon the final equilibrium of a hemolytic system, how would such an explanation fit the known facts concerning the rate at which hemolysis occurs in very strongly hypotonic solutions? It is entirely conceivable that in such solutions the rate of osmotic hemolysis might be affected either by a slowing of the rate of entrance of water or by a slowing,—though not a prevention—of the escape of hemoglobin by a delay in the attainment of the proper condition of the pores, or by a combination of both factors. Since it is unlikely, with a rate of increase of cell volume as rapid as that in distilled water, that the delay

in the escape of hemoglobin would be very great, it seems entirely possible that at least a part of the observed effect of narcotics on the rate of hemolysis by water may be due to an actually decreased rate of penetration of this substance. It is to be noted, however, that the possible effect must in any case be rather slight.

In this connection it is of interest to consider the work of Siebeck (1922) on the effect of narcotics on the rate of passage of ions between the erythrocyte and its surroundings and also that of Anselmino and Hoenig (1930) on the entrance of the non-electrolytes erythritol, arabinose, xylose, etc. In the former case, actual chemical analyses were made at several intervals and it is therefore virtually certain that the permeability of the cell to the substances in question was dealt with directly. In the work of Anselmino and Hoenig, though the methods were not quite so direct, it is also very likely that their interpretation of their results as indicating a production by narcotics of a decreased permeability to various slowly penetrating non-electrolytes is correct. It is perhaps significant that the decrease in permeability to ions and to rather slowly penetrating non-electrolytes is much greater than any decrease for water that could be inferred from the present experiments. If the "pore" theory were correct, it would be expected that the hypothetical diminution of the pore diameter produced by narcotics would exert an effect upon permeability which would become proportionately greater as the size of the molecule increased. The water molecule, being the smallest of those commonly supposed to enter the erythrocyte in this manner, would be affected least of all.

It should be emphasized that this explanation of the manner in which narcotics may conceivably affect osmotic hemolysis is suggested merely as a convenient working hypothesis. Its chief advantages are that it explains in essentially the same manner both "rate" and "equilibrium" effects and that, as far as we are aware, it is not incompatible with any known facts. It is by no means necessary, however, that the rate and equilibrium effects should be explained in the same way; in the case of temperature, for example (Jacobs, 1928), they seem almost certainly to be of a different nature. It is entirely possible that at any time facts may come to light with which the present theory is inconsistent; in that case it may readily be abandoned without greatly changing the significance of the experimental data here presented.

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SUMMARY

1. The observation of previous investigators that narcotic substances in proper concentrations tend to oppose osmotic hemolysis is confirmed in the case of several urethanes.

2. It is shown that the conclusion frequently drawn from such observations, that the antihemolytic effect of narcotics is due to a decreased permeability of the erythrocyte to water, is unwarranted by the existing experimental evidence. The necessity for a separation of "rate" and "equilibrium" factors in studies on osmotic hemolysis is emphasized.

3. It is shown by experiments in which these factors are properly separated that a slight but measurable retardation of osmotic hemolysis may be produced by low concentrations of urethanes. The possible nature of the mechanism of this retardation, which may perhaps in part involve a decreased permeability of the cell to water, is discussed.

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